A COMPARATIVE STUDY

OF THE

TOXIN PRODUCTION OF DIPHTHERIA BACILLI.

BY

THEOBALD SMITH, M.D., AND ERNEST L. WALKER.

1897.

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INTRODUCTION.*

By Theobald Smith, M.D.

This inquiry was suggested by the following important problems bearing upon the restriction of diphtheria: —

1. Is there any difference in the pathogenic power of diphtheria bacilli from different localities?

2. Is the pathogenic activity of bacilli producing diphtheria in the summer season different from that of those producing disease in winter?

3. Is there any reduction in the pathogenic power of bacilli in cases in which they persist in the throat after recovery?

4. Are there any differences noticeable between the bacilli of mild and those of severe cases?

The third and the fourth questions have been attacked by other observers, while the first and the second have not been especially investigated. The answers to the third and the fourth questions have been, as a rule, negative. Observers have found little or no difference in bacilli from mild and severe cases, nor have they been able to show any recognizable loss of virulence in the bacilli persisting in the throat after recovery.

The reasons for entering upon this subject again were the opportunity we have had of examining cultures from different towns within the State, and more especially certain improved methods of cultivation by which the maximum toxin-producing capacity of each bacillus could be brought out and measured more accurately than had been done heretofore.

The selection of cultures for the study of the questions stated above has not been entirely satisfactory, mainly because much of the clinical information necessary to a proper choice was not accessible

* The writer wishes to acknowledge the faithful assistance of J. R. Stewart, to whom the preparation of the culture media was chiefly entrusted. As will be seen from what follows, this is not a simple task.

at the time the cultures were received, and in some instances obtainable only with difficulty at the last moment when the final results were tabulated. We hope, however, that the material at hand may be supplemented by more in the near future.

The Mode of Action of Diphtheria Bacilli.

It is now a generally accepted theory that diphtheria bacilli act in the main through the toxins which they produce, and which are rapidly diffused into the fluids containing the vegetating bacilli. The contents of the bacilli themselves seem to be of little moment as pathogenic factors. Park and Williams * allowed the washed diphtheria bacilli to soak for a week "in a 0.5 per cent. alkaline carbolic solution." The injection of one cubic centimeter did not "produce any marked reaction in a 500-gramme guinea-pig," although the bacilli themselves were powerful toxin-producers. Kossel † collected the bacillar membranes from cultures, washed the bacilli repeatedly by centrifugalizing with 0.5 per cent. sodium chloride; then, after killing them with vapors of chloroform, he extracted them for several days in a few cubic centimeters of weakly alkaline fluids. The extract was only feebly poisonous, for it required 5 cubic centimeters to kill a 360-gramme guinea-pig in forty-eight hours.

Brieger and Boer ‡ found that shaking diphtheria bacilli with ammonium chloride and allowing them to stand for eighteen to twenty hours removes the toxin from the bodies of the bacilli. The bacilli after extraction were fatal to a 500-gramme guinea-pig, in doses of 0.01 gramme of bacillar substance. They acted by producing local necrosis. Brieger states that antitoxin had no effect upon this action of the dead bacilli, and that immunization towards it by gradually increasing doses failed. The poison itself withstood an hour's boiling.

These experimental observations, taken together, show that the toxin in the culture fluid and not the body substance of the bacilli themselves is to be looked upon as the disease agent. The success following the prompt application of antitoxin in sufficient doses is an additional support to this view. Moreover, the bacilli themselves do not penetrate into the body in large numbers, hence need not be specially considered as adding to the toxic effect of their products.

* "Journal of Exp. Med.," I., page 174. † "Centralblatt f. Bakteriologie," XIX., page 977.
‡ "Deutsche Med. Wochenschrift," 1896.

We may, for convenience, regard the disease-producing power of diphtheria bacilli as made up of two elements, — toxicity and virulence. The former represents the rate of accumulation of toxin in culture fluids, and is easily measured; the virulence, on the other hand, which may be regarded as the behavior of diphtheria bacilli toward living tissue, is as yet an unknown quantity. This distinction between the toxic product of diphtheria bacilli and their inherent vital power to cope with living tissue seems to be established, at least experimentally, by the increase in virulence of diphtheria bacilli in their passage through a series of guinea-pigs, which has been reported by various observers. Thus, Aronson * states that a culture which was at first fatal to guinea-pigs of medium weight, in 0.1 cubic centimeter doses, was fatal, after some serial inoculations, in doses of 0.008 cubic centimeter. That is to say, its virulence was augmented twelve times. This experiment evidently means not that the toxin formed in the sub-cutis of guinea-pigs became twelve times stronger in quality at the end of the series, but that the bacilli injected were capable, by an adaptation of some sort, to multiply much more abundantly toward the end of the series, and hence produce more toxin. The other explanation, that the toxin itself had become more potent in quality, could only gain confidence if the bouillon culture produced much more toxin at the end of the experiment than at the beginning, the conditions remaining precisely the same.

To compare the disease-producing power of diphtheria bacilli from different sources, it was, therefore, thought best to study the relative accumulation of toxin in bouillon, and eliminate the bacilli by filtration before the test upon animals. The writer is fully aware of the fact that but an instrument of pathogenic power is here dealt with, and under artificial conditions, since we do not know the nature of the nutritive fluid which the bacilli make use of on mucous membranes, nor, as a consequence, whether the toxin production in bouillon is a true index of the production of toxin on mucous membranes. The problem is, in fact, very complex, as with all infectious diseases, and all we can hope to do at a time is to examine one factor of disease as carefully as possible, while eliminating all the others for the time being. The use of living cultures upon animals is of no service in these experiments, because it introduces at once three variable factors: (1) the bacilli as potential toxin-producers after

* "Berl. klin. Wochenschr.," 1893, Nos. 25 and 26.

injection; (2) the poison of their bodies after destruction; and (3) the toxin pre-formed in the culture fluid injected. As a consequence, all who have used cultures find them uncertain in their action, as compared with the toxin alone. The bacilli injected as nearly free from fluid as possible are equally unreliable as measures of toxicity, as the following tests show: —

Two cultures of diphtheria bacilli are selected, which differ considerably in toxin-producing power, as is shown in Table I., where the toxin-producing power of one (No. 14) is about three times that of the other (No. 40). Inclined agar cultures are prepared from each, and after six days' growth the bacilli are removed with a platinum wire, the amount of moist bacilli weighed and stirred in 5 cubic centimeters sterile bouillon, making a moderately cloudy suspension. One cubic centimeter contained by weight about 0.0007 grammes of moist bacilli.

Bacillus No. 14. — Five-tenths cubic centimeter of the suspension, injected subcutaneously into a guinea-pig weighing 313 grammes, is fatal in five days; 1 cubic centimeter is fatal to a 330-gramme pig in six days.

Bacillus No. 40. — Of the suspension made in the way described, 1 cubic centimeter is injected into a guinea-pig weighing 315 grammes. Animal just escapes death, and is chloroformed on the sixth day. Another, weighing 330 grammes, receives 0.5 cubic centimeter. A slough forms at the place of injection. The guinea-pig remains in fair condition.

Though these tests show a greater activity on the part of Bacillus No. 14, yet we miss here not only the sharp definition in the results obtained by varying the dose of the same culture, but also in comparing the effect of the same doses of cultures from different sources.

A prolonged study of the relative production of toxin in bouillon under certain uniform conditions has shown such remarkably uniform results with the same culture, even after long intervals of time, that the results obtained in this way may be accepted as showing an inherent difference in the various bacilli studied.

The Method employed in comparing the Toxin Production of Different Cultures.

In a former publication* the writer has given the conditions which must be fulfilled in order that a maximum accumulation of toxin may take place in bouillon cultures. The facts there considered and

* "Trans. Association American Physicians," for 1896.

others since then brought out may be very briefly reviewed here. In 1895 Spronck * called attention to the fact that the variable amount of sugar present in beef was responsible for the great fluctuations observed in the toxicity of diphtheria cultures. The writer had observed this independently of Spronck, by studying the relation between the amount of toxin in cultures and the amount of sugar as determined by the fermentation test. Sugar is present in all beef, but in perhaps 10 per cent. the amount is very small. In bouillon made from such beef the writer obtained very strong toxin. In bouillon from beef containing over 0.1 per cent. sugar the toxin was very feeble.

The cause for this difference lies in the acid or acids formed from the dextrose by the diphtheria bacillus, which inhibit the multiplication in a direct ratio to the amount formed. In sufficient quantity the growth may be entirely checked, and finally, when the acidity has reached a certain degree, the bacilli and the toxin are destroyed. Whether there are other causes at work besides mere inhibition of multiplication remains undetermined.

A small amount of dextrose, up to 0.05 per cent., is not inimical to toxin production; in fact, it seems to be more favorable than none at all, probably because a certain minimum amount is necessary for the cell life of the diphtheria bacilli. Bearing these facts in mind, we are better able to comprehend the various changes going on in cultures. The life of the culture begins with a rapid multiplication of the bacilli introduced and the formation of a surface membrane usually within twenty-four hours. At the same time, any sugar present is acted upon at once, with the result that the reaction becomes more acid. If the acidity increases beyond 2 per cent. of a normal acid solution,† the culture is likely to become languid, the surface membrane rifted and settle to the bottom. Some bacilli, by a vigorous surface growth which probably oxidizes the acid products formed, may subdue a larger amount of acid, even to 3.5 per cent., and cause a rapid return towards the alkaline level. The toxin appears in greatest concentration when the alkaline level has been reached, usually within eight to twelve days when sugar is present in small amount only. When sugar is more abundant the acid period is prolonged, during which little growth is evident. After

* "Annal. de l'Institut Pasteur," 1895, page 758.

† *I. e.*, each 100 cubic centimeters of the culture fluid requires 2 cubic centimeters of a normal solution of alkali to bring the whole to the neutral point as determined by phenolphthallein.

several weeks a slow alkalizing tendency brings the culture to a more vigorous growth and to an alkaline reaction, but without much accumulation of toxin.

Without going into more detail on this subject, we may summarize the conditions under which diphtheria bacilli produce maximum amounts of toxin in the ordinary 1 per cent. (Witte) peptone bouillon as follows: —

1. Muscle sugar in the fluid from 0 to 0.05 per cent.

2. Initial reaction from 0.8 to 1.5 per cent. normal acid, the lower figure pertaining to bouillon containing the largest amount of sugar, the higher to bouillon containing none.

3. A thin layer of bouillon freely exposed to the air through one or more cotton-plugged openings in the vessel, and quiescent because the surface membrane which forms within twenty-four hours must not be disturbed.

4. The accumulation of toxin should be permitted to go on until the growth is checked by the alkaline reaction. This appears in from eight to twelve days, according to the initial reaction and amount of sugar present, and the growth ceases when the reaction is equivalent to 0.2 to 0.3 per cent. normal alkali.

The main difficulty before us, therefore, is to get beef containing only traces of dextrose. The writer's original plan, to select the bouillon in accordance with the fermentation test, is not feasible, because so little can be used. Spronck's suggestion, to allow the beef to lie for several days, in order that a partial decomposition by bacteria may transform the sugar, is better, but suffers from certain difficulties. The kind of bacteria cannot be controlled, and frequently the sugar is found but partially removed. Latterly, the writer has given up this method for one more rapid and certain in its action. The beef infusion is prepared either by extracting the chopped beef at 60° C. for several hours, or over night in the refrigerator. After removal of the beef the infusion is inoculated with a culture of some bacterium which rapidly acts upon dextrose, and placed in the thermostat over night. The writer has tried only B. coli, and found a complete transformation of carbohydrates taking place over night.

In the case of bouillon designed for diphtheria toxin the incubation should be as short as possible, so as to leave a trace of sugar in the fluid. This can be accomplished by placing the inoculated infusion in the thermostat at 10 P.M. and removing early next morn-

ing (8 A.M.). The infusion is then made up in the usual way, with 1 per cent. peptone, ½ per cent. sodium chloride. The final reaction should range, according to the amount of sugar left as stated above, between 0.8 and 1.5 per cent. normal acid, phenolphthallein being used as indicator. It can easily be brought to any desired point by adding from sterile solutions the calculated amount of normal acid or alkali (HCL or NaHO). The whole procedure is very simple after it has been put into routine practice. At any rate, the bacteriologist must make up his mind to give up the early slovenly methods of preparing culture media, or else be prepared for constant reverses and failures.

The bouillon must be sterilized finally in the autoclave, since the ordinary steaming frequently fails to destroy certain spore-bearing anaërobes, which begin to multiply after the diphtheria bacilli have formed a membrane and deoxidized the culture medium. These anaërobes inhibit the production of toxin.*

Park and Williams claim† that the amount of dextrose in beef purchased in New York City is not sufficient to interfere with the maximum accumulation of toxin if the culture be made sufficiently alkaline to begin with. This claim I cannot support by my experience with beef bought in the Boston markets. It may be that these authors had under observation bacilli which had acquired, through surface cultivation, a greater power to promptly oxidize acid products. This power is not possessed, as a rule, by bacilli recently isolated from the throat, with which this article deals.

A number of observers have published studies of the relative virulence of diphtheria bacilli from various sources, and those persisting in the throat after recovery for a variable length of time. It is not the object of this article to re-examine these writings and review the results obtained. For a summary of the literature the reader is referred to the article by J. H. Wright in the "Boston Medical and Surgical Journal," Vol. 131 (1894), page 329, and "Scientific Bulletin" No. 1 of the health department, city of New York (1895). A perusal of the various articles will show that the method of testing the virulence of the diphtheria bacilli was not adapted to give uniform or quantitative results. Thus, Park and Beebe, on page 23 of the

* Since writing this, it has been observed that high temperatures in the autoclave may modify the bouillon in such a manner that only little toxin is formed subsequently. This matter is now under investigation.

† "Journal of Experimental Medicine," I. (1896), page 164.

bulletin referred to, recommend alkaline glucose bouillon as a culture medium, and the injection of cultures forty-eight hours old. Wright used sugar bouillon very largely. From what we now know of the inhibitory and destructive action of the acids formed from dextrose by diphtheria bacilli, the use of more than 0.1 per cent. dextrose in bouillon must be considered as at least unsafe. However, the authors followed general usage at that time, for even Escherich, in his work on diphtheria issued in 1894 (page 91), states that dextrose is not decomposed in appreciable manner by diphtheria bacilli, and therefore has no influence on growth.

Authors have not, so far as the writer knows, reported comparative tests of toxin production under conditions as nearly uniform as possible. It was mainly to fill this gap, if possible, that the series of cultures to be described were subjected to a comparative examination from the point of view of toxin production. Table I. gives a condensed account of the work done upon which the calculation of toxin production rests. In this table will be found: (1) the amount of acid produced in dextrose bouillon; (2) the condition of the bouillon used for the cultures; and (3) the test of the filtrate on guinea-pigs. The acid production will be dealt with farther on. The facts relative to the bouillon used need some explanation.

The beef used for bouillon, with one exception, was allowed to decompose according to Spronck's suggestion, but the results were not uniform, as stated above. In some of the bouillon the dextrose was absent, in some present in traces, in some in more appreciable amount, according to tests made with the fermentation tube and B. coli. In none was it present in the amount usually found in bouillon made from fresh beef. It is not probable that this slight fluctuation in the amount of dextrose had any appreciable influence on the culture. Where a doubtful result was obtained it was usually supplemented later on with a second test.

The question might be asked, Why not use the same bouillon for all bacilli studied, in place of the many lots actually employed? This would seem the simplest procedure, provided the bouillon did not change with time under the influence of light and air. A diminution in the amount of toxin produced in bouillon which had been standing for some time in a closet not absolutely dark had been casually observed. It is probable that bouillon in vacuo and kept in a dark place might meet the conditions of the problem, but bouillon kept under ordinary conditions would not. Further investigations

are now in progress to determine more precisely the degree of change produced in bouillon by age.

It might be claimed that different bacilli isolated from the throat would have different rates of growth in bouillon, and that the accumulation of toxin was simply a factor of the rate of multiplication, rather than of any inherent differences in the physiology of the bacilli. To answer this claim a determination of the number of diphtheria bacilli in cultures is not trustworthy, for the reason that diphtheria bacilli clump together, and the number of colonies in plate cultures may not indicate the number of bacilli used in preparing the plate. Again, bacilli may die in the course of growth, and others take their places. The writer has therefore endeavored to estimate the vigor of growth by the amount of change in the reaction produced. Cultures which in a given time in the same bouillon produce nearly the same amount of alkali may be regarded as having performed the same amount of work and grown with equal vigor. The uniformity of reaction in the various groups of bacilli studied together, after ten or twelve days, was such as to leave little doubt that the growth had been equally vigorous. When any culture lagged perceptibly behind, it was usually repeated with other bouillon.

The extent of the alkali production varies with the initial reaction of the bouillon and the presence of dextrose. Cultures containing the latter became at first more acid before swinging back to alkalinity. In Table I., therefore, it was deemed best to give both the initial reaction of the bouillon, the approximate amount of dextrose and the final reaction. Some idea may thus be gained of the amplitude of change which the fluid underwent during the period of growth permitted.

The culture vessel used at first was a large test tube placed in an inclined position after inoculation. This was soon given up for the Erlenmeyer flask, in which the depth of the bouillon was about 1.5 centimeters.

The toxin formed after ten to twelve days was tested upon guinea-pigs. The fluid was passed through filter paper until clear, then diluted with sterile salt solution, so that the quantity of toxin injected was contained in 1 cubic centimeter. Usually 0.1 cubic centimeter of toxin was injected. The place of injection chosen was the left side of the abdomen. Great care was exercised to deposit the fluid in the subcutis, and not to prick the muscles of the abdominal

wall. A vascular injection of the omentum or peritoneum is usually a result of the introduction of some of the fluid into the abdomen. When such reddening was noted at the autopsy, the test was repeated upon another animal, since death is hastened somewhat when this occurs. Guinea-pigs weighing between 300 and 350 grammes were used whenever possible. When larger ones had to be used, the increase in weight was duly taken into account.

From the results of such inoculations the minimum fatal dose upon a guinea-pig weighing 300 grammes was calculated. The calculation when such had to be made was based upon the fact that the minimum fatal dose usually kills a guinea-pig in from three and one-half to six days. If x represents this dose, then a guinea-pig which succumbed in two and one-half days, or sixty hours, received $\frac{10}{9}x$, and one which succumbed in thirty-six hours, $\frac{20}{9}x$. Guinea-pigs of greater weight do not necessarily bear an exact equivalent increase of toxin, but usually somewhat less. In general, it may be said that the values given as the minimum fatal doses may err within 10 per cent., owing to various factors which cannot be controlled. Among these is a slight variation among guinea-pigs in their tolerance of the virus, the darker (black, or black and red) animals being able to stand about 10 per cent. more toxin than the white animals. Even if we allow a variation of 10 per cent. in the values given in Table I., the general outcome of the comparative study is not made in any sense untrustworthy.

Comparative Study of Forty-two Cultures of Diphtheria Bacilli and of Four Cultures of Pseudo-Diphtheria Bacilli from Different Localities in Massachusetts.

By Theobald Smith and E. L. Walker.

Morphology.

The following description of the morphology and the staining peculiarities of the bacilli studied is based on microscopic preparations from cultures of twenty-four hours' growth at 35° to 37° C. on Löffler's blood-serum mixture, uniformly fixed and stained. The cover-slip preparations were dried in open air at room temperature, fixed by heating twenty minutes in a dry-air sterilizer at the temperature of 120° C., and stained eight minutes with Löffler's alkaline methylene blue solution. It may be remarked, however, that experiment shows that the method of fixation has little if any effect on the outline of the bacillus or upon the aggregation of its chromatin, and consequently upon the irregularity of its staining.

In length the diphtheria bacilli vary from 1.5 μ to 13 μ, and for the purpose of description it is convenient to distinguish three groups: short bacilli, including all bacilli under 2 μ in length; bacilli of medium length, including all bacilli between 2 μ and 4.5 μ; and long bacilli, including all bacilli over 4.5 μ in length. Bacilli in culture No. 33 are rather remarkable for their length, averaging 7.5 μ to 10 μ, while a few were found as long as 13 μ.

It may be said of diphtheria bacilli in general that there appears to be a tendency for the shorter bacilli to become swollen at the middle and for the long bacilli to become swollen at the ends; and that the short bacilli are usually straight, while the long bacilli are usually curved or bent at an obtuse angle.

Comparison on the basis of length, outline and manner of staining allows the bacilli of the forty-two virulent cultures to be divided into three types, of which the following description may be given: —

Type I. — Bacilli of medium length, straight, cylindrical or slightly swollen in the middle, with blunt ends, and with intensely stained granules in an otherwise uniformly but less deeply stained cell. In the shorter bacilli of this type these granules are usually situated at the ends of the rod, one at each end; but in the longer bacilli there may be, in addition to these polar granules, one or more interpolar granules. These deeply stained bodies are usually of less diameter than the thickness of the bacillus, but may be of greater diameter, swelling the bacillus at the points where they are situated.

Type II. — Bacilli long, slender, curved, more or less swollen at one or both ends, and with alternating stained and unstained (or faintly stained) cross-segments.

Type III. — This includes seven of the forty-two cultures. Bacilli are of various lengths, swollen in the middle, with tapering ends, and with broad, unstained terminal and intermediate segments. These unstained terminal segments may be so extensive that a body simulating a nucleus in the middle of the cell is the only stained portion. More often the cell may consist of two stained and three unstained cross-bands. The staining of this type differs from that of Type II., in that the alternating segments of Type II. are narrow and numerous and the terminal ones are always stained.

Modifications of these types and intermediate forms occur even among bacilli of the same culture, but in nearly every case one form predominates sufficiently to allow the culture to be ranged under one of these three types. In the routine work of bacteriological diagnosis of diphtheria, as carried on under the direction of the State Board of Health, Type I. and its modifications are found in about 90 per cent. of the positive cases and bacilli of Type II. make up the greater part of the other 10 per cent. Bacilli of Type III. are very infrequently found. This classification holds good for young cultures on Löffler's serum mixture only.

Bacilli belonging to these three types have so far proved virulent to guinea-pigs when tested according to the methods given in another part of the text. But besides these a certain number of bacilli (Nos. 3, 4, 39 and 44 of the tables) have been isolated which are non-pathogenic, and which belong to the class of pseudo-diphtheria bacilli described farther on.

Toxin-producing Power.

The toxicity of the culture fluid of the forty-six cultures after an incubation at 35° C. for ten to twelve days ranged as follows, the 300-gramme guinea-pig being the basis of the computations: —

	Cubic Centimeters.
In one the minimum fatal dose is	.036–.04
In one the minimum fatal dose is	.045
In five the minimum fatal dose is	.050
In five the minimum fatal dose is	.060
In four the minimum fatal dose is	.070
In four the minimum fatal dose is	.075
In eleven the minimum fatal dose is	.080
In two the minimum fatal dose is	.090
In four the minimum fatal dose is	.100
In five the minium fatal dose is	.120
In four no toxin was formed.	

Leaving aside for the moment the non-pathogenic forms, we notice in this summary, first of all, a considerable uniformity in the toxin-producing power. It is true the strongest toxin producer accumulates three times as much toxin as the weakest, but only one of such strength was found. It will be noticed also that the greater number of bacilli studied produce an 0.08 cubic centimeter toxin. If we group the cultures as follows, —

	Cultures.
.036–.06 cubic centimeter toxins,	12
.070–.09 cubic centimeter toxins,	21
.100–.12 cubic centimeter toxins,	9

the predominance of the middle group is better brought out.

Cultures of much greater toxin-producing power have been isolated by Park and Williams. Of these, the minimum fatal dose is reported to range from 0.002 to 0.01 cubic centimeter. It is not stated whether these cultures produced this amount of toxin at the outset, or after periods of artificial cultivation.

By comparing these figures with the results of earlier observers, the greater efficiency of the method described appears in striking relief. Experimenters when first preparing antitoxin had some difficulty in finding bacilli whose toxin would yield a minimum fatal dose of 0.08 to 0.1 cubic centimeter. In the series here recorded only five out of forty-two fell below this mark.

Although the clinical records of the cases from which the bacilli came are very meagre, they suffice to show that any direct relation

between toxin production and severity of the disease is not obvious. This has been the inference of observers before us (Wright, Park and very recently Timaschew *), and we are able to confirm it after the application of more uniform and exact methods. This is what might be expected when we contemplate the complex nature of the disease process, the many factors which may enter into it, both on the part of the patient and the invading bacilli. There is one factor, for instance, which may modify the course of the disease, and therefore make any present-day estimates untrustworthy, — namely, antitoxin. If applied early enough, it may convert a potentially serious case into a mild one, in spite of a virulent organism. Antitoxin was used in nearly every case from which bacilli were studied, but the time of administration and the number of units injected were not reported excepting in a few cases, so that the facts on hand are not worth any serious study. All that can be said is that the toxin-producing power of bacilli from mild and from severe cases varies but little, and that all throat affections must be regarded equally dangerous if diphtheria bacilli are present.

The Toxin-producing Power of Bacilli persisting in the Throat after Recovery.

Much interest has been aroused by the patients in whose throats diphtheria bacilli may be found a variable length of time after subsidence of all symptoms of disease. Löffler, in his investigation of the ætiology, found diphtheria bacilli in the throat of a healthy child. Roux and Yersin first called attention to the persistence of diphtheria bacilli after recovery, but they disseminated the impression that there was a gradual attenuation going on which eventually made them harmless. That this may be true in certain cases is not disputed, otherwise it would be difficult to account for the presence, in the mouth of some healthy persons, of bacilli in no way distinguishable from those associated with disease processes except by an absence of virulence.† This attenuation has not been observed by subsequent investigators, however, and no reliance can be placed upon it to purge the throat of the recovered case of its infectious character.

Among the forty-six cultures studied there were eleven made from the throat fifteen to sixty-two days after the appearance of the dis-

* "Centralblatt f. Bakteriologie," XXI. (1897), page 623.

† Park and Beebe, *loc. cit.*, page 37.

ease. Owing to the meagre records returned, it is impossible to state how long *after* the subsidence of the symptoms the bacilli were obtained from the throat; but by a reference to Table II., where the relative severity of each case is noted, some idea may be gained by the reader of the probable duration. The following table summarizes these cases. It includes two from which harmless pseudo-forms were obtained: —

Number of Culture.	Date of Earliest Symptoms.	Date of Culture.	Interval (in Days).	Minimum Fatal Dose of Toxin (Cubic Centimeters).
23,	July 12, 1896.	Aug. 3, 1896.	22	.07
24,	July 14, 1896.	Sept. 9, 1896.	57	.08
26,	Aug. 28, 1896.	Oct. 19, 1896.	52	.05
27,	Sept. 27, 1896.	Oct. 19, 1896.	22	.06
34,	Oct. 22, 1896.	Nov. 17, 1896.	26	.05
36,	Nov. 15, 1896.	Nov. 30, 1896.	15	.08
39,	Nov. 22, 1896.	Dec. 29, 1896.	37	Not toxic.
40,	Dec. 18, 1896.	Jan. 4, 1897.	17	.12
42,	Dec. 31, 1896.	Jan. 16, 1897.	16	07
43,	Feb. 20, 1897.	March 19, 1897.	27	.08
44,	Feb. 8, 1897.	March 25, 1897.	45	Not toxic.
45,	Feb. 9, 1897.	March 28, 1897.	42	.08
46,	Jan. 29, 1897.	April 1, 1897.	62	.08

If we exclude the harmless, non-toxic cultures (Nos. 39, 44), which will be discussed farther on, we observe that, so far as toxin production is concerned, the length of time the bacilli have sojourned in the throat has no tendency to reduce it below the average. This is still better brought out by arranging the cultures in the following groups: —

Group.	Days after Beginning of Disease.	Number of the Culture.	Toxicity (Cubic Centimeters).
I.,	15 to 20	35	.08
		40	.12
		42	.07
II.,	20 to 30	23	.07
		27	.06
		34	.05
		43	.08
III.,	50 to 62	24	.08
		26	.05
		45	.08
		46	.08

Still more to the point are cultures Nos. 22 and 23, which were isolated from the same case, one three, the other twenty-two, days after the onset of the disease. Here the toxin production was practically the same for both cultures.

Pseudo-Diphtheria Bacilli.

From the table it will be seen that four of the forty-six cultures isolated were found to be pseudo-diphtheria bacilli. It does not lie within the scope of this paper to discuss at length the relation between the true diphtheria bacillus and the pseudo-diphtheria bacillus. A very good discussion will be found in the work of Park and Beebe, to which the reader is referred. Since its appearance nothing new has been added to this subject. These bacilli, however, influence to a certain degree the interpretation of problems in public sanitation, so that a brief reference to them becomes necessary.

These bacilli, generally known as pseudo-diphtheria bacilli, are short rods (1.5 μ to 3 μ), with rounded or tapering ends (often oval in culture), and uniformly stained, or with a single narrow, unstained cross-segment. A few cylindrical, pear and hour-glass shaped bacilli are occasionally seen; but involution forms are not marked, even in old cultures. They are distinguished from diphtheria bacilli by being shorter, smaller, more uniform in size, shape and manner of staining, and, as pointed out by Escherich, by a tendency to lie parallel in cover-slip preparations. These bacilli are of occasional occurrence, both in the throats of patients suffering from non-diphtheritic throat affections and in true diphtheria mingled with the Klebs-Löffler bacilli. They are, however, almost always present in small numbers, while the diphtheria bacilli, in recent cases, are usually present in large numbers and well differentiated. It is only in convalescent cases of long duration that the pseudo-diphtheria bacilli are likely to cause doubt. They might be mistaken for the last few remaining diphtheria bacilli, or the reverse might occur. A few remaining virulent forms may be regarded as pseudo-forms. Diphtheria bacilli directly from the membrane from the throat, or from cultures scarcely at all developed, sometimes resemble quite closely the pseudo-diphtheria bacilli in morphology and staining.

The morphological differences are reinforced by at least two biological differences of importance, — the absence of any power to produce acids in bouillon containing dextrose, and the lack of

pathogenic power. In Table I. it will be seen that all toxin-producing bacilli, when multiplying in bouillon containing 1 per cent. dextrose, produce a considerable amount of acid, ranging from 3.5 to 5 per cent. of a normal acid solution when phenolphthallein is used as an indicator. A few cultures were found which produce between 5 and 6 per cent. The pseudo-diphtheria bacilli produced no acid under the same circumstances. The culture slowly becomes more alkaline, as shown in the table (Nos. 3, 4, 39, 44). The culture fluid of these bacilli was likewise free from toxin. Guinea-pigs which received from six to twelve times the average fatal dose of the virulent cultures showed no trace of infiltration at the place of injection and no loss in weight.

Though there are these three distinctive features of pseudo-diphtheria bacilli, — characteristic morphology, absence of acid and of toxin production, — it is not a simple matter to recognize them as such promptly under the microscope when taken from throat cultures, unless the observer has had considerable training. It is highly probable, therefore, that Roux and Yersin in their earlier work may have mistaken pseudo-diphtheria bacilli for true diphtheria bacilli, when they found virulent and non-virulent forms together in the throats of convalescents. This may explain their at that time quite natural position, — that the virulent forms were being transformed into non-virulent forms. In two of the cases tabulated above (Nos. 39 and 44) the pseudo-diphtheria bacilli were isolated respectively thirty-seven and forty-five days after the beginning of the disease. Here, without a more profound study of the cultures, the belief might gain the upper hand that the cultures represented diphtheria bacilli which had lost their virulence. This position can no longer be upheld, and we must accept or at least act upon the presumption that the pseudo-diphtheria bacilli belong to a wholly different group of bacilli, and that a loss of pathogenic power of the genuine forms does not take place in the mouth for months after the subsidence of the disease, when such forms persist after recovery.

Of the non-virulent but otherwise characteristic diphtheria bacilli, described by Park and Beebe and by others more recently, none have come under observation.

Table I.

Designation of Bacillus.	Acid Production in 1 Per Cent. Dextrose Bouillon. (See p. 665.)	Toxin Production in Bouillon.					Toxin Tested on Guinea-pigs.				Minimum Fatal Dose (calculated in Cubic Centimeters).
		Designation of Bouillon.	Quantity of Muscle Sugar (Per Cent.).	Mode of Cultivation.	Original Reaction. (See p. 655.)	Final Reaction.	Weight of Animal (Grms.).	Dose of Toxin, Subcutaneous (Cubic Centimeters).	Date of Test.	Result of Test.	
1,	5.35	90	Trace	Inclined test tube,	−1.00	+.10	396	.10	May 29, 1896,	Dies in 8 days,	.080
		90	Trace.	Inclined test tube,	−1.00	−.16	345	.10	June 11, 1896,	Slough,	.120
	3.89	90	Trace.	Inclined test tube,	−1.00	+.30	302	.10	July 6, 1896,	Dies in 16 days,	.100
		112	Trace.	Inclined test tube,	−1.10	+.00	315	.10	July 6, 1896,	Dies in 2¾ days,	.080
		193	.02-.04	Flask,	−.90	+.10	300	.10	Feb. 25, 1897,	Dies in 1⅞ days,	.060
2,	5.39	90	Trace.	Inclined test tube,	−1.00	+.10	380	.05	May 29, 1896,	Slough,	-
		193	.02-.04	Flask,	−1.10	+.20	300	.10	Feb. 25, 1897,	Dies in 1½ days,	.050
3,	No acid.	90	Trace.	Inclined test tube,	−1.00	+.10	380	.50	May 29, 1897,	No effect; non-pathogenic,	-
4,	No acid.	90	Trace.	Inclined test tube,	−1.00	+.10	370	.50	May 29, 1897,	No effect; non-pathogenic,	-
5,	3.76 4.41	90	Trace.	Inclined test tube,	−1.00	−.15	395	.10	June 11, 1896,	Dies in 6½ days,	.080
6,	4.14	90	Trace.	Inclined test tube,	−1.00	−.15	380	.10	June 11, 1896,	Dies in 2¼ days,	.075
		115	Trace.	Inclined test tube,	−1.10	+.00	293	.10	Aug. 12, 1896,	Dies in 2 days,	.075
7,	4.58	90	Trace.	Inclined test tube,	−1.00	−.35	380	.10	June 11, 1896,	Dies in 4¼ days,	.080
8,	3.56 4.19	90	Trace.	Inclined test tube,	−1.00	−.23	300	.10	June 29, 1896,	Slough,	.120
9,	4.37	90	Trace.	Inclined test tube,	−1.00	−.22	285	.08	June 29, 1896,	Slough,	.120
10,	4.50	90	Trace.	Inclined test tube,	−1.00	−.32	325	.10	June 29, 1896,	Dies in 8 days,	.100

11, . .	4.25	90	Trace.	Inclined test tube,	—1.00	—.46	315	.10	June 29, 1896,	Slough, ...120
12, . .	3.85	90	Trace.	Inclined test tube,	—1.00	—.21	310	.10	June 29, 1896,	Dies in 2 days, . . .075
	3.93	115	Trace.	Inclined test tube,	—1.10	+.00	455	.10	Aug. 12, 1896,	Dies in 3½ days, . . .070
	4.89	140	.05	Flask, . . .	—1.40	+.00	515	.10	Nov. 19, 1896,	Dies in 1½ days, . . .040
		176	None.	Flask, . . .	—1.10	+.20	335	.05	Jan. 28, 1897,	Dies in 2½ days, . . .045
13, . .	4.17	112	Trace.	Inclined test tube,	—1.10	+.10	304	.10	July 22, 1896,	Dies in 2⅙ days, . . .080
14, . .	3.91	112	Trace.	Inclined test tube,	—1.10	—.10	308	.10	July 22, 1896,	Dies in 1½ days, . . .040
	3.94	115	Trace.	Inclined test tube,	—1.10	—.05	312	.10	Aug. 12, 1896,	Dies in 1½ days, . . .040
		140	.05	Flask, . . .	—1.40	—.68	502	.10	Nov. 19, 1896,	Dies in 1⅚ days, . . .040
		176	None.	Flask, . . .	—1.10	+.00	315	.05	Jan. 28, 1897,	Dies in 1¾ days, . . .040*
		176	None.	Flask, . . .	—1.10	+.00	360	.05	Feb. 4, 1897,	Dies in 2½ days, . . .040
		176	None.	Flask, . . .	—1.10	+.00	360	.10	Feb. 4, 1897,	Dies in 1½ days, . . .040
		297	Trace.	Flask, . . .	—1.55	+.00	316	.04	Mar. 30, 1897,	Dies in 2½ days, . . .036
15, . .	3.95	115	Trace.	Inclined test tube,	—1.10	—.10	309	.10	Aug. 12, 1896,	Dies in 3½ days, . . .090
16, . .	4.16	112	Trace.	Inclined test tube,	—1.10	—.13	309	.10	July 22, 1896,	Dies in 2 days, . . .075
17, . .	4.31	112	Trace.	Inclined test tube,	—1.10	—.20	308	.10	July 22, 1896,	Dies in 2 days, . . .075
18, . .	3.78	115	Trace.	Inclined test tube,	—1.10	+.00	313	.10	Aug. 12, 1896,	Dies in 6 days, . . .100
19, . .	4.07	115	Trace.	Inclined test tube,	—1.10	+.00	310	.10	Aug. 12, 1896,	Slough, ...120
20, . .	Lost.	115	Trace.	Inclined test tube,	—1.10	+.00	313	.10	Aug. 12, 1896,	Dies in 1⅚ days, . . -*
		129	Trace.	Inclined test tube,	—.70	+.00	370	.10	Oct. 9, 1896,	Dies in 2½ days, . . .080
21, . .	4.28	115	Trace.	Inclined test tube,	—1.10	—.15	308	.10	Aug 12, 1896,	Dies in 4½ days, . . .100

* Injection intra-abdominal, by accident.

TABLE I. — *Concluded.*

Designation of Bacillus.	Acid Production in 1 Per Cent. Dextrose Bouillon. (See p. 665.)	Toxin Production in Bouillon.					Toxin Tested on Guinea-pigs.				Minimum Fatal Dose (calculated in Cubic Centimeters).
		Designation of Bouillon	Quantity of Muscle Sugar (Per Cent.).	Mode of Cultivation.	Original Reaction. (See p. 655.)	Final Reaction.	Weight of Animal (Grms.).	Dose of Toxin, Subcutaneous (Cubic Centimeters).	Date of Test.	Result of Test.	
22,	4.19	115	Trace.	Inclined test tube,	−1.10	−.05	315	.10	Aug. 12, 1896,	Dies in $1\frac{5}{6}$ days,	.070
		176	None.	Flask,	−1.10	+.20	300	.10	Feb. 17, 1897,	Dies in $2\frac{1}{8}$ days,	.080
23,	4.46	129	Trace.	Inclined test tube,	−.70	−.20	374	.10	Oct. 9, 1896,	Local swelling,	–
		140	.05	Flask,	−1.40	−.64	520	.10	Nov. 19, 1896,	Dies in 3 days,	.070
		176	None.	Flask,	−1.10	+.20	300	.10	Feb. 17, 1897,	Dies in 2 days,	.075
24,	4.83	129	Trace.	Inclined test tube,	−.70	+.00	372	.10	Oct. 9, 1896,	Dies in $2\frac{1}{2}$ days,	.080
25,	4.75	140	.05	Flask,	−1.40	−1.15	580	.10	Nov. 19, 1896,	Dies in 3 days,	.050
		193	.02-.04	Flask,	−.90	−.05	300	.10	Feb. 25, 1897,	Dies in $1\frac{1}{2}$ days,	.050
26,	4.86	140	.05	Flask,	−1.40	−.27	540	.10	Nov. 19, 1896,	Dies in 2 days,	.050
27,	4.65	140	.05	Flask,	−1.40	−.38	530	.10	Nov. 19, 1896,	Dies in $2\frac{1}{2}$ days,	.060
28,	5.88 6.31	140	.05	Flask,	−1.40	−.54	500	.10	Nov. 19, 1896,	Dies in $2\frac{1}{2}$ days,	.060
29,	4.72	159	.05-.08	Flask,	−1.20	−.74	520	.13	Dec. 26, 1896,	Slough,	.100
30,	4.32	159	.05-.08	Flask,	−1.20	−.38	615	.13	Dec. 26, 1896,	Dies in 10 days,	.070
31,	4.74	159	.05-.08	Flask,	−1.20	−1.35	630	.13	Dec. 26, 1896,	Slough,	–
		176	None.	Flask,	−1.10	+.10	300	.10	Feb. 13, 1897,	Dies in $1\frac{5}{6}$ days,	.070
32,	(?)	151	Trace.	Flask,	(?)	−.08	430	.10	Dec. 10, 1896,	Dies in $2\frac{1}{2}$ days,	.070

33, . .	4.42	159	.05-.08	Flask, . . .	−1.20	−1.25	590	.13	Dec. 26, 1896,	Slough, ...	.080 (?)
		193	.02-.03	Flask, . . .	−.90	+.10	300	.10	Feb. 25, 1897,	Dies in 1½ days, . .	.050
34, . .	4.73	159	.05-.08	Flask, . . .	−1.20	−1.00	540	.13	Dec. 26, 1896,	Dies in 2 days, . . .	.050
		176	None.	Flask, . . .	−1.10	+.20	330	.05	Feb. 17, 1897,	Dies in 11¾ days, . .	0.50
35, . .	4.87	159	.05-.08	Flask, . . .	−1.20	−2.33	660	.13	Dec. 26, 1896,	Slough, ...	-
		176	None.	Flask, . . .	−1.10	+.00	325	.10	Feb. 17, 1897,	Dies in 2¾ days, . .	.090
36, . .	4.37	176	None.	Flask, . . .	−1.10	+.10	410	.10	Jan. 26, 1897,	Dies in 4½ days, . .	.080
37, . .	4.00	176	None.	Flask, . . .	−1.10	+.10	380	.10	Jan. 26, 1897,	Dies in 1¾ days, . .	.060
38, . .	4.23	176	None.	Flask, . . .	−1.10	+.30	360	.10	Jan. 26, 1897,	Dies in 1¾ days, . .	.060
39, . .	.32	176	None.	Flask, . . .	−1.10	+.00	550	1.00	Jan. 26, 1897,	No effect, ...	-
40, . .	4.79	176	None.	Flask, . . .	−1.10	+.20	370	.10	Jan. 26, 1897,	Slough, ...	-
		176	None.	Flask, . . .	−1.10	+.20	350	.15	Feb. 4, 1897,	Dies in 2½ days, . .	.120
41, . .	4.23	176	None.	Flask, . . .	−1.10	+.20	410	.10	Jan. 26, 1897,	Dies in 6½ days, . .	.080
42, . .	4.14	176	None.	Flask, . . .	−1.10	(?)	470	.10	Jan. 26, 1897,	Dies in 2½ days, . .	.070
43, . .	5.20	226	Faint trace.	Flask, . . .	−1.30	−.15	384	.10	Apr 27, 1897,	Dies in 2½ days, . .	.080
44, . .	.55	226	Faint trace.	Flask, . . .	−1.30	−.55	404	1.00	Apr. 27, 1897,	No effect, ...	-
45, . .	4.83	226	Faint trace.	Flask, . . .	−1.30	−.00	312	.10	Apr. 27, 1897,	Dies in 2½ days, . .	.080
46, . .	4.67	226	Faint trace.	Flask, . . .	−1.30	−.75	307	.16	Apr. 27, 1897,	Dies in 1½ days, . .	.080

Table II.

Designation of Culture.	Locality.	Age of Patient (Years).	Date of Earliest Symptoms.	Date of Culture.	Nature of Case.	Antitoxin Used.	Termination of Case.	Minimum Fatal Dose of Toxin on Guinea-pigs (Cubic Centimeters).	Remarks.
1	Everett, .	1½	April 19, '96,	April 21, '96,	Severe, membranous croup.	No,	Death, .	.060	Bacilli of medium length, cylindrical or slightly swollen in the middle, and with deeply stained granules.
2	Everett, .	3	April 27, '96,	April 27, '96,	Mild, with laryngeal symptoms.	Yes,	Recovery,	.050	Bacilli of medium length, rather thick, cylindrical or slightly swollen in the middle, and with deeply stained granules.
3	Fitchburg, .	23	May 8, '96,	May 11, '96,	Scarlet-fever, . .	No,	Recovery,	No effect.	Bacilli short (about 2 μ), with rounded or tapering ends, and with a single unstained or faintly stained cross-segment.
4	Everett, .	2½	May 10, '96,	May 12, '96,	Moderately severe, .	Yes,	Death, .	No effect.	Bacilli short (from 1.5 μ to 2 μ), oval, evenly stained, or with a single very narrow unstained cross-segment.
5	S. Braintree, .	5	May 11, '96,	May 16, '96,	Severe, with laryngeal symptoms.	Yes,	Death, .	.080	Bacilli of various lengths, with tapering ends or variously swollen, and with large unstained terminal and intermediate segments.
6	E. Saugus, .	7	May 20, '96,	May 22, '96,	Well-marked case, with laryngeal symptoms	Yes,	Recovery,	.075	Bacilli rather below medium length, considerably swollen in the middle, many being with tapering ends, and with large unstained terminal and intermediate segments.
7	Everett, .	8	May 19, '96,	May 20, '96,	Severe, ...	Yes,	Recovery,	.080	Bacilli of medium length, with tapering ends, and with unstained terminal and intermediate segments; a few cylindrical forms with deeply stained granules
8	E. Somerville,	7	May 25, '96,	May 25, '96,	Mild, ...	Yes,	Recovery,	.120	Bacilli rather long, slender, cylindrical or with slightly swollen ends, and with deeply stained polar granules.
9	Somerville, .	8	May 24, '96,	May 26, '96,	Mild, ...	No,	Recovery,	.120	Bacilli of medium length, or long bacilli, that are cylindrical or with slightly swollen ends and with alternating stained and unstained cross-segments.
10	Watertown, .	19	May 28, '96,	May 28, '96,	Severe, with laryngeal symptoms.	(?)	Recovery,	.100	Bacilli of medium length, with tapering ends, and with a stained nucleus like body in an otherwise colorless cell.
11	Roslindale, .	29	May 30, '96,	June 4, '96,	Severe, with laryngeal symptoms.	Yes,	Recovery,	.120	Bacilli of medium length, straight, cylindrical or slightly swollen in the middle, and with deeply stained polar granules.
12	E. Somerville,	7	June 4, '96,	June 6, '96,	Severe, ...	Yes,	Recovery,	.040	Bacilli of medium length, or long bacilli, that are cylindrical or with slightly swollen ends, and with alternating stained and unstained cross-segments
13	Somerville, .	18	June 9, '96,	June 10, '96,	Mild, ...	Yes,	Recovery,	.080	Bacilli rather short, plump, with tapering ends, and with unstained terminal and intermediate segments.
14	Hyde Park, .	9¾	June 15, '96,	June 16, '96,	Severe, ...	Yes,	Recovery,	.036	Bacilli rather long, slender, curved, cylindrical or with slightly swollen ends, and with alternating stained and faintly stained cross segments.
15	Everett, .	3½	June 15, '96,	June 16, '96,	Severe, with laryngeal symptoms.	Yes,	Recovery,	.090	Bacilli of medium length, cylindrical or slightly swollen in the middle, and mostly evenly stained.

16	Somerville, .	3½	June 16, '96,	June 17, '96,	Rather severe, . .	Yes,	Recovery,	.075	Bacilli of medium length, slightly swollen in the middle, and with faintly stained ends and intermediate segments.
17	Everett, .	17	June 23, '96,	June 24, '96,	Severe, ...	Yes,	Recovery,	.075	Bacilli of various lengths, slender, with tapering ends or with swollen ends, and with broad unstained cross-segments.
18	Winchester, .	17	July 1, '96,	July 4, '96,	Mild, ...	Yes,	Recovery,	.100	Bacilli of medium length, or long bacilli, that are slender, cylindrical or slightly swollen in the middle or irregularly swollen, and with unstained ends and one or more intermediate segments.
19	Somerville, .	16	July 2, '96,	July 6, '96,	Mild, ...	Yes,	Recovery,	.120	Bacilli rather below medium length, cylindrical or slightly swollen in the middle, and with deeply stained granules.
20	Salem, . .	7	July 2, '96,	July 6, '96,	Severe, ...	Yes,	Recovery,	.080	Bacilli short, small, mostly swollen in the middle, with tapering ends, and with faintly stained terminal and intermediate segments.
21	Everett, .	13	July 6, '96,	July 6, '96,	Mild, ...	Yes,	Recovery,	.100	Bacilli of medium length, or long bacilli, that are slender, cylindrical or with swollen ends, and with alternating stained and unstained cross-segments
22	S. Hanover, .	11	July 12, '96,	July 15, '96,	Well-defined case, .	Yes,	Recovery,	.070	Bacilli long, curved, with more or less swollen ends, and with alternating deeply stained and faintly stained cross-segments.
23	S. Hanover, .	11	July 12, '96,	Aug. 3, '96,	From same patient as No. 22.	–	–	.070	Bacilli rather long, slender, curved, with pronounced swelling at the ends, and with alternating deeply stained and faintly stained cross-segments.
24	Somerville, .	3	July 14, '96,	Sept. 9, '96,	Very mild, . . .	Yes,	Recovery,	.080	Bacilli of medium length, straight, cylindrical, and with deeply stained granules.
25	Somerville, .	5	Oct. 11, '96,	Oct. 13, '96,	Severe, with laryngeal symptoms.	Yes,	Recovery,	.050	Bacilli of medium length, or rather long bacilli, that are straight, cylindrical, and with deeply stained polar granules.
26	Norwell, .	8	Aug. 28, '96,	Oct. 19, '96,	Severe, ...	Yes,	Recovery,	.050	Bacilli of medium length, straight, cylindrical or slightly swollen in the middle, and with deeply stained granules.
27	Salem, . .	10	Sept. 27, '96,	Oct. 19, '96,	Very severe, . .	Yes,	Recovery,	.060	Bacilli of medium length, cylindrical or with somewhat pointed ends, and with deeply stained granules.
28	Danvers, .	7	Oct. 20, '96,	Oct. 28, '96,	Mild, ...	Yes,	Recovery,	.060	Bacilli long, slender, curved, cylindrical or more or less swollen at the ends, and with alternating stained and unstained cross-segments.
29	W. Everett, .	11	Oct. 14, '96,	Oct. 24, '96,	Mild, ...	Yes,	Recovery,	.100	Bacilli of medium length, straight, mostly cylindrical, and with deeply stained granules.
30	Watertown, .	8	Oct. 26, '96,	Oct. 27, '96,	Condition not given, .	Yes,	Recovery,	.070	Bacilli long, cylindrical, or slightly swollen at the ends, and with thickly alternating stained and unstained cross-segments.
31	Salem, . .	3	Oct. 30, '96,	Oct. 31, '96,	– –	–	–	.070	Bacilli rather short (1.9 μ), straight, slender, cylindrical or slightly swollen in the middle, and with deeply stained granules.
32	W. Medford,	12	Nov. 7, '96,	Nov. 7, '96,	Mild, ...	Yes,	Recovery,	.070	Bacilli of medium length, or short bacilli, that are straight, slender, cylindrical, and with deeply stained granules.
33	Medford, .	2	Nov. 15, '96,	Nov. 17, '96,	Severe, with laryngeal symptoms.	Yes,	Recovery,	.050	Bacilli very long, very slender, with slightly swollen ends, and with alternating stained and unstained cross-segments.
34	Taunton, .	6	Oct. 22, '96,	Nov. 17, '96,	Mild, ...	Yes,	Recovery,	.050	Bacilli of medium length, very slender, cylindrical, and with deeply stained granules.

TABLE II. — *Concluded.*

Designation of Culture.	Locality.	Age of Patient (Years).	Date of Earliest Symptoms.	Date of Culture.	Nature of Case.	Antitoxin Used.	Termination of Case.	Minimum Fatal Dose of Toxin on Guinea-pigs (Cubic centimeters).	Remarks.
35	Watertown,	17	Nov. 26, '96,	Nov. 27, '96,	Moderately severe,	Yes,	Recovery,	.090	Bacilli of medium length, very slender, straight, cylindrical, and with deeply stained granules.
36	Beverly,	2	Nov. 15, '96,	Nov. 30, '96,	Severe, with laryngeal symptoms.	Yes,	Recovery,	.080	Bacilli of medium length, mostly straight, cylindrical or slightly swollen in the middle, and with deeply stained polar granules.
37	Beverly,	19	Nov. 16, '96,	Nov. 21, '96,	Severe,	Yes,	Recovery,	.060	Bacilli of medium length, or short bacilli (1.5 μ to 3 μ), that are mostly straight, cylindrical or slightly swollen in the middle, and with deeply stained granules.
38	No. Adams,	$\frac{3}{4}$	Dec. 23, '96,	Dec. 24, '96,	Severe, with laryngeal symptoms.	Yes,	Recovery,	.060	Bacilli of medium length, cylindrical or slightly swollen at the ends, and with alternating stained and unstained cross-segments.
39	Watertown,	6	Nov. 22, '96,	Dec. 29, '96,	Severe,	Yes,	Recovery,	No effect.	Bacilli rather short, with rounded or tapering ends, and with a single unstained cross segment.
40	Beverly,	10	Dec. 18, '96,	Jan. 4, '97,	Severe, with laryngeal symptoms.	Yes,	Recovery,	.120	Bacilli rather long, slender, curved, more or less swollen at the ends, and with alternating deeply stained and faintly stained cross-segments.
41	Chelsea,	3	Dec. 31, '96,	Jan. 6, '97,	Laryngeal symptoms,	Yes,	Recovery,	.080	Bacilli long, slender, curved, more or less swollen at the ends, and with alternating stained and unstained cross-segments.
42	Lexington,	16	Dec. 31, '96,	Jan. 16, '97,	Mild,	Yes,	Recovery,	.070	Bacilli of medium length, straight, slender, cylindrical, and with deeply stained granules
43	Hingham,	53	Feb. 20, '97,	Mar. 19, '97,	Mild,	Yes,	Recovery,	.080	Bacilli of medium length, or long bacilli, that are mostly cylindrical, and with deeply stained granules.
44	Beverly,	18	Feb. 8, '97,	Mar. 25, '97,	Mild,	Yes,	Recovery,	No effect.	Bacilli short, regular, and evenly stained or with a single unstained cross-segment.
45	Danvers,	18	Feb. 9, '97,	Mar. 23, '97,	Mild,	Yes,	Recovery,	.080	Bacilli long, rather thick, cylindrical or slightly swollen at the ends, and with alternating deeply stained and faintly stained cross-segments.
46	Danvers,	26	Jan. 29, '97,	April 1, '97,	Severe,	Yes,	Recovery,	.080	Bacilli rather long and large, mostly slightly swollen in the middle, and with deeply stained granules.